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Characterization of a Buried Latosol from Northern Puerto Rico

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INTRODUCTION

It is an accepted fact in pedology that soils with fully developed profiles existed long before today's soil mantle was formed. Most of these ancient soils have disappeared through the process of erosion; however, in certain places the soils were buried by rock materials and their profiles remain intact. These buried profiles may well serve as a key to the study of the role that past interactions among climatic and geologic processes played in soil formation.

Buried soils have, since long, been a subject of investigation by soil scientists (*3, 4*).[2] Recently Kaye (*1*) observed the occurrence of buried profiles along the northern coast of Puerto Rico.

Kaye observed that "West of Arecibo, in a series of interesting road cuts and excavations, . . . [porous sandstones of aeolian origin] can be seen deposited against the eroded slopes cut into the marine sands and limestones. Between the two types [of rocks, sandstone, and limestone] is a well-developed soil zone consisting of a red sandy loam" (*1*). He further observed a similar buried soil, some 1 to 4 feet in depth, interbedded between two sandstones in a hill west of the mouth of the Manatí river and that other exposures of the same type occurred as far east as Carolina. According to Kaye, in some places the Islote (*5*) soils could be traced as mere outcrops of these buried soils. He claimed that the Islote soils, whether buried or in outcrops, could be traced to a period of low sea level during one of the Pleistocene glacial stages and that probably the origin of the soils dated from a pre-Wisconsin glacial stage. Based on his geologic observations Kaye presented an

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[2] Italic numbers in parentheses refer to Literature Cited, p. 126.

interesting hypothesis propounding that laterization processes acted with more effectiveness in the past than they do at the present.

OBJECTIVE

There is a need of more detailed studies of buried profiles; these may provide information on the significance of past time as a factor in soil formation. Although it may help toward a better understanding of the paleopedology of Puerto Rico, this paper is merely a first approach to the subject. Herein are reported some morphological, physical, and chemical data on a buried soil profile exposed in a road cut in northern Puerto Rico; comparisons are made between the properties of the buried profile and those of its purported outcrops, an Islote soil nearby.

MATERIALS AND METHODS

Morphological studies were conducted on road-cut exposures in State Road No. 2, west of the city of Arecibo, in the northern coast of the Island. Information was secured as to number of horizons, thickness, color (based on moist field conditions and using the Munsell color-chart standards), and apparent texture and structure. Samples were taken from each horizon for analyses in the laboratory. (See Fig. 1.)

Undisturbed soil cores (3″ x 3″) were taken from each horizon by using an Uhland sampler. Hydraulic-conductivity measurements were made by recording the rate at which water moved through a column of saturated soil at a known head. Quick drainage was determined by placing the saturated soil core on a Büchner funnel at a 60-cm. tension and measuring the water drained out in 15 minutes. Water removed and retained at pF 1.7 was measured by bringing the soil to equilibrium in a 60-cm. tension table, and determining the variation in weight of the core. Water remaining at pF 2.7 and at pF 4.2 was determined by submitting saturated soil samples to pressures of ½ and 15 atmospheres, respectively, in special chambers and plates. The maximum saturation was calculated using the data obtained from the above-described moisture determinations.

The soil cores were dried at 110° C. and the bulk density of each sample was computed by dividing the net dry weight of each soil core by its original volume. The pore space was determined by assuming that the soil specific gravity was 2.65.

Mechanical analysis was done by the pipette method, using centrifugation instead of filtration through a Chamberlain filter. The pH was determined electrometrically by means of a Macbeth pH-meter. Total nitrogen was determined by the standard Kjeldahl method and the organic matter by chromic acid titration. The cation-exchange capacity was determined by the ammonium acetate method.

EXPERIMENTAL RESULTS

SOIL MORPHOLOGY

Profiles of a buried soil were examined in a road cut near Km. 78 H. 9, Road No. 2. The slope of this buried soil was estimated to be approximately 30 or 35 percent. Over the top layer of soil there is a mass of porous calcareous sandstone, deposited in a cross-bedded fashion, and some 18 to 25 feet in height. One of the most striking features of this buried soil

FIG. 1.—View of the buried latosol in a roadcut at the outskirts of Arecibo.

profile is the red color of the solum that grades into the shattered yellower sandstone. Numerous iron concretions throughout the solum are also a rather prominent feature of this profile. Thickness of the A and B horizons is variable, ranging from 1 to 4 feet. Medium to coarse structure predominates in the A horizon, the aggregates being rather small. The B horizon tends to be more firm and tight. The C horizon consists of a nonaggregated shattered sandstone rock material.

A typical profile of this buried latosol can be described as follows:

A. 0–21″ Red (2.5 *YR* 4/8) loamy sand with a moderately well-developed structure and cleavage planes in all directions. The aggregates

are rather small, almost rounded, $\frac{H}{V} = 1$, with medium to coarse internal porosity. Numerous iron concretions are present.

B. 21–42″ Red (2.5 *YR* 4/6) sandy clay; probably the tightest layer in the profile. Weakly developed structure, almost massive with predominantly horizontal cleavage. The aggregates are medium in size, rounded to subangular, $\frac{H}{V} > 1$, with medium to coarse internal porosity. Many large iron concretions are present together with many water-worn pebbles.

C. 42–80″ Brownish-yellow (10 *YR* 6/6) unaggregated sandstone of marine origin with black streaks throughout.

MECHANICAL COMPOSITION

This soil is a loamy sand; there is, however, some clay accumulation particularly in the C horizon as compared to the A and B as shown in table 1.

PHYSICAL PROPERTIES

Table 2 gives the results of hydraulic-conductivity measurements and other soil-moisture studies conducted on a number of undisturbed soil cores collected at the buried-profile site. Hydraulic conductivity was moderately slow in the A horizon, slow in the following horizon, and moderately slow through the desintegrated parental sandstone. Quick-drainage values (water drained at 15-minutes under a 60-cm. tension) followed the same pattern as the hydraulic conductivities. Maximum saturation decreased from nearly 40 percent at the upper 21 inches to 3[illegible] in the next layer, but increased to 42.5 in the sandstone.

Table 3 presents data on bulk density and total porosity. Bulk-density values were all high, approaching 1.90 gm./cc. in the 21 to 42″ layer, the tightest of the profile. Total-porosity values were larger where bulk-density values were smaller. They ranged from approximately 30 percent in the tight subsoil to over 40 percent in the parent material.

CHEMICAL PROPERTIES

The results of the chemical analyses of the various horizons of this buried soil are shown in table 4. The pH values indicate alkalinity, which decreased slightly with depth. This soil was exceedingly low in organic matter throughout the profile. Nitrogen levels were very low, too. The cation-exchange capacity of the topsoil can be ranked as low, but there were moderate increases when penetrating through the subsoil to the weathered sandstone.

TABLE 1.—*Mechanical analysis of a buried latosol derived from sandstone in northern Puerto Rico*

Horizon	Depth	Sand (>0.02 mm.)	Silt (0.02–0.002 mm.)	Clay (<0.002 mm.)
	Inches	*Percent*	*Percent*	*Percent*
A	0–21	80.0	14.6	5.4
B	21–42	75.4	18.5	6.1
C	42+	76.0	14.0	10.0

TABLE 2.—*Hydraulic-conductivity and other soil-moisture data obtained from a buried latosol in northern Puerto Rico*

Horizon	Depth	Hydraulic conductivity	Water drained at 60 cm. in 15 minutes	Maximum saturation	Water removed at pF 1.78	Water remaining at—		
						pF 1.78	pF 2.7	pF 4.2
	Inches	*In./hr.*	*Percent*	*Percent water*	*Percent*	*Percent*	*Percent*	*Percent*
A	0–21	0.97	6.51	39.80	5.79	31.81	4.25	2.42
B	21–42	.14	3.18	34.00	2.24	31.25	6.74	4.41
C	42+	.30	5.51	42.50	3.33	38.39	8.16	4.13

TABLE 3.—*Bulk density and porosity for each horizon of a buried latosol in northern Puerto Rico*

Horizon	Depth	Bulk density	Porosity
	Inches	*Gm./cc.*	*Percent*
A	0–21	1.74	34.56
B	21–42	1.88	29.65
C	42+	1.44	40.51

TABLE 4.—*Mean pH, organic matter, nitrogen, and cation-exchange capacity for each horizon of a buried latosol in northern Puerto Rico*

Horizon	Depth	pH	Organic matter	Nitrogen	Cation exchange capacity per 100 gm. of dry soil
	Inches		*Percent*	*Percent*	*M.e.*
A	0–21	8.2	0.057	0.010	3.8
B	21–42	7.6	.092	.010	7.2
C	42+	7.2	.092	.004	8.4

DISCUSSION

The morphological studies pointed to the striking, well-defined red coloring, dominating the soil above the brownish-yellow unconsolidated parental sandstone. This coloring is indicative of good oxidation and con-

centration of iron, which indicates that laterization must have been a very active process at the time this soil was developed.

The low fertility level of this soil, as revealed by low cation-exchange capacity, and low organic-matter and nitrogen levels, further confirm the possibility that laterization was the process responsible for the genesis of this soil.

If the available water is regarded to be the difference between water remaining in the soil when tensions in the vicinity of pF 1.78 develop, and water remaining when permanent wilting occurs (pF 4.2), this soil could hold slightly above 10 acre-inches of water for crops in the upper 3 feet. Most of this water, however, would drain slowly under gravity, because practically all of it would be held at tensions below ½ atmosphere (pF 2.7).

The study of the physical properties of this soil is rather revealing. The fact that a very high percentage of the water held at pF 2.7 is unavailable, *i.e.*, held at tensions approaching pF 4.2, marks another similarity between this buried soil and other exposed latosols. Lugo-López (*2*) has shown that latosols approach the permanent wilting percentage, even when they contain relatively large amounts of water. This accounts for their relative droughtiness, usually observed under field conditions.

The moderate to slow water-conducting ability of this soil, in contrast to normal latosols of Puerto Rico, may be attributed to the compacting effect of the 18- to 20-foot layer of sandstone overlying the profile. The very high bulk-density values further indicate this compaction effect. Because of the sandy nature of the soil material many large pores remained unaffected as shown by the water retained and removed at pF 1.78. Most of these pores could be drained easily with further tensions up to pF 2.7.

Comparisons with data from Islote soils (fig. 2) outcropping about 3 km. west of the profile studied indicate similar cation-exchange capacity and about a tenfold increase in organic-matter and nitrogen levels in the outcropping soil. The pH of the buried soil is slightly higher than that of the outcropping soil. This increased alkalinity could be ascribed to the leaching of lime from the overlying highly calcareous sandstone.

Following the same comparison, but restricting it to the upper 21 inches of soil, the less dense soil was the outcropping one; it was more porous, but many more of its pores were coarse, draining easily and quickly, even at pF 1.78.

The subsoil of the outcropping Islote, however, was tight, dense, with but a small percentage of water being drained at low tensions. It was very similar to the buried profile subsoil in morphological, physical, and chemical properties, excepting perhaps the above-mentioned higher organic-matter and nitrogen levels.

From the foregoing observations this buried Islote soil profile could be classified as a latosol. So far, evidence has been provided herein indicative of the activity of laterization in past geologic periods. The interesting question raised by Kaye (*1*), as to whether this soil-forming process was more active in the past than at present remains yet to be answered.

FIG. 2.—An Islote soil outcropping near the buried latasol.

SUMMARY

Data on the morphological, physical, and chemical properties of a buried soil profile in northern Puerto Rico are presented. The solum is of a distinct red color some 42 inches in depth, grading into a brownish-yellow unaggregated sandstone of marine origin and containing numerous iron concretions.

The soil is a loamy sand corresponding rather closely to the Islote soil, a somewhat extensive soil in northern Puerto Rico. The relatively high

water retention at pF 4.2 in comparison to that retained at pF 2.7 indicates the probable droughtiness of this soil, a feature of most latosols in Puerto Rico. The rather relatively low fertility status—low exchange capacity, organic matter, and nitrogen—is also characteristic of latosols. The alkalinity throughout the profile may partly be the result of lime leaching over a period of years from the overlying 18- to 25-foot layer of highly calcareous sandstone. The weight of this sandstone layer can also account for the compaction of the solum.

The over-all picture indicates that laterization was an active process in past geologic time, such as in the Pleistocene epoch when this soil was probably developed.

RESUMEN

Se presentan aquí datos sobre las propiedades morfológicas, físicas y químicas de un suelo sepultado en el norte de Puerto Rico. El suelo y subsuelo son rojos, con numerosas concreciones de hierro, con una profundidad media de unas 42 pulgadas, y descansan sobre material de piedra arenisca no consolidado, de origen marino y de color amarillo pardo.

El suelo es arenoso lómico y corresponde a los de la serie Islote que están extensamente distribuídos por la costa norte. La humedad retenida a tensiones en la vecindad del punto de marchitez permanente (pF 4.2) es bastante alta en relación a la retenida a pF 2.7, cosa característica de suelos lateríticos en Puerto Rico. Su fertilidad relativamente baja, indicada por una baja capacidad de intercambio y bajos niveles de materia orgánica y nitrógeno, es también característica de suelos lateríticos. La alcalinidad observada a través de todo el perfil puede deberse en parte a la lixiviación de compuestos de calcio de la capa de piedra arenisca carbonatada de 18 a 25 pies de grosor que sepulta este perfil. El peso de esta roca también ha causado gran compacción del suelo y subsuelo.

En general, se puede deducir que el proceso de laterización estuvo activo durante épocas geológicas pasadas, como en el Pleistoceno, cuando este suelo probablemente se formó.

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The Effect of Synthetic Soil Conditioners on Soil-Aggregate Stability and the Production of Potatoes and Stringless Beans

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INTRODUCTION

During the past 5 years considerable attention has been given to the use of synthetic soil conditioners to improve the stability of soil aggregates and hence the allied physical properties of problem soils.

The soil conditioners are either calcium or sodium salts of organic polymers molecularly related to synthetic rubber and fibers. Either a hydrolyzed polyacrylonitril polymer (HPAN) or a modified vinyl acetate maleic acid (VAMA) compound has been the basis of most of the formulations now on the market.

The information now available indicates that the more commonly known conditioners will stabilize soil aggregates but will not form them (*5*)[2]. Some method of fragmenting a dense soil must be used in order to obtain beneficial effects from the stabilizing chemical. The physical conditions usually attained with sound seedbed-preparation techniques can be stabilized longer when the conditioners are effectively used. Widespread use of these chemicals in general farming is at present limited by their cost.

Until price reductions are feasible and more information can be obtained as to response of soils and crops and persistence in the soil, their use will have to be highly specialized, *i.e.*, in flowerbeds, home gardens, nurseries, and as a research tool. As it is possible that the establishment of production facilities and rising competition may effect price reductions, field and laboratory studies were initiated in Puerto Rico to evaluate the use of synthetic soil conditioners as a means of increasing crop production through improvement of soil structure.

MATERIALS AND METHODS

The experimental field was located near the town of Aibonito in the highlands of Puerto Rico. The soil has been classified as a Juncos clay, a Gray-Brown Podzolic medium-deep soil of the uplands, associated with the extensive, rather steep Múcara soils, but owing to its smoother relief it has a thicker surface soil and greater depth to parent rock. It is derived from massive tuffs, tuffaceous shales, and other volcanic rocks.

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[2] Italic numbers in parentheses refer to Literature Cited, p. 133.

Juncos clay has a 7-inch dark grayish-brown plastic, sticky, heavy-clay surface soil. It is underlain by a 14- to 20-inch layer of yellowish-brown, compact, plastic, sticky clay. Below this is a 10-inch layer consisting of disintegrated rock and fine-grained yellowish-brown silty clay loam. Below a depth of 30 to 40 inches are the tuffaceous rocks (*6*). Mineralogical studies of the soil reveal a very high content of feldspars, mostly albite and andesine, with traces of orthoclase (*3*). Montmorillonite predominates in the upper 8-inch layer with some kaolin, but in the 8- to 15-inch layer there is some illite in addition to the other two clay minerals. The exchange capacity reaches 58 m.e. per 100 gm. of dry soil with a clay content of 26 percent.

The experiment was laid out following a balanced incomplete-block design with 10 treatments, including control untreated plots, replicated 6 times in a 15-block arrangement. The plots were 6 by 4 feet in size. The soil conditioners used were formulations 6 and 9 of Krilium[3], dry powder, 100-percent active ingredient, and Aerotil[4], wettable flakes, 83-percent active ingredient. Each chemical was used at three rates: 900, 1,800, and 3,600 pounds to the acre.

The conditioners were applied on January 18, 1954, and carefully mixed with the uppermost 6 inches of soil. The check plots received the same mechanical treatment as the treated plots to minimize any possible differences other than those imposed by the treatments. Before the application of the conditioners the soil was given a preventive treatment with Aldrin at a rate of 1 quart in 50 gallons to the acre for the control of worms and mole crickets.

Potatoes of the Kennebeck variety were planted on January 20, 1954, in rows 2 feet apart, with seven plants to the row. A 10–6–14 fertilizer was applied at a rate of 1,000 pounds to the acre. The potatoes were harvested on April 14, 1954.

Stringless beans of the Bountiful variety were planted in the same plots on April 19, 1954. A 9–10–5 fertilizer was applied at a rate of 800 pounds to the acre. The beans were harvested between June 10 and 24, 1954. Three pickings were necessary to harvest the whole crop. Both the potato and the bean crops were irrigated by sprinkling whenever required.

At the time of harvesting the bean crop, soil samples were taken from each plot. Aggregate stability was determined in the 2- to 1-mm. size according to the method proposed by Brant, Bendixen, and Slater (*1*). This procedure requires that the aggregates be soaked for 2 minutes followed by

[3] Trade name for a number of organic polymers developed by the Monsanto Chemical Co., St. Louis 4, Mo.

[4] Trade name for the chemical supplied by the American Cyanimid Co., 30 Rockefeller Plaza, New York 20, N. Y.

wet screening for 15 minutes. The aggregates remaining in the 1-mm. sieve were oven-dried at 105° C. and percentage stability was determined by dividing the oven-dried weight by the weight of aggregates used originally.

EXPERIMENTAL RESULTS

SOIL-AGGREGATE STABILITY

Table 1 presents the data obtained on aggregate stability of the soils under various stabilizing treatments. The untreated soils had nearly 67 percent of water-stable aggregates. In general, the percentage of stable aggregates increased to over 73 to 78 at the lower level of conditioner treatment, 75 to nearly 87 at the intermediate level, and from almost 84 to 91 at the high level. At all levels formulation 6 of Krilium was the most effective soil-aggregate stabilizing compound. There were significant and even highly significant differences between the stability of soil aggregates in the check plots and in all the conditioner-treated plots, except in those where Krilium 9 was applied at the minimum level. The over-all differences were significant at the 1-percent level.

TABLE 1.—*Water-stable aggregates in Juncos clay as affected by treatment with 3 soil conditioners used at 3 levels*

Treatment identification No.	Treatment		Water-stable aggregates
	Conditioner	Rate per acre	
		Lb.	*Percent*
1	Check	—	66.9
2	Krilium 9	3,600	87.9
3	do.	1,800	78.0
4	do.	900	73.4
5	Krilium 6	3,600	91.1
6	do.	1,800	85.7
7	do.	900	78.1
8	Aerotil	3,600	83.5
9	do.	1,800	75.7
10	do.	900	75.4
L.S.D. at the 5-percent level			7.1
L.S.D. at the 1-percent level			9.5

The influence of synthetic soil-conditioning chemicals on the structural conditions of Juncos clay is shown graphically in figure 1.

POTATO YIELDS

Data on the yields of potatoes from untreated and soil-conditioner-treated plots are given in table 2. The over-all differences are significant,

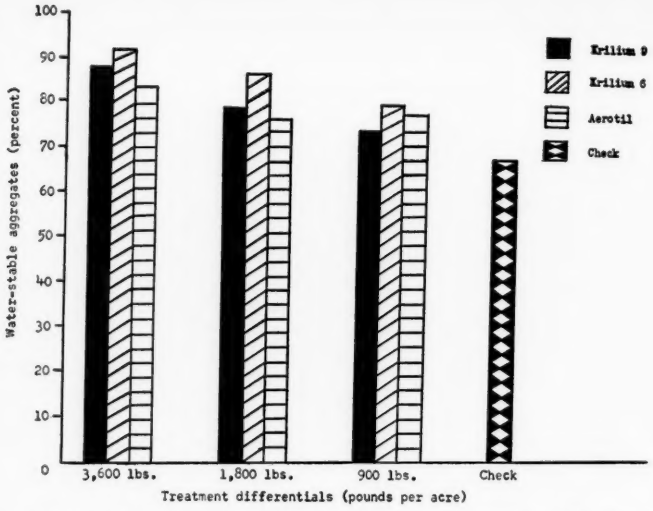

FIG. 1.—Effect of various soil conditioners at three rates of application on the stability of soil aggregates.

TABLE 2.—*Yield of potatoes on Juncos clay as affected by treatment with 3 soil conditioners used at 3 levels*

Treatment identification No.	Treatment		Yeld of indicated size per acre			Total
	Conditioner	Rate per acre	Large (over 2" diam.)	Medium (2"-1½" diam.)	Small (under 1½" diam.)	
		Lb.	*Cwt.*	*Cwt.*	*Cwt.*	*Cwt.*
1	Check	—	2.7	7.5	0.9	11.1
2	Krilium 9	3,600	4.4	9.7	1.5	15.6
3	do.	1,800	4.6	9.1	1.1	14.8
4	do.	900	5.4	6.7	1.1	13.2
5	Krilium 6	3,600	5.5	8.4	1.1	15.0
6	do.	1,800	5.7	6.1	1.2	13.0
7	do.	900	2.9	8.8	1.3	13.0
8	Aerotil	3,600	4.1	8.3	1.2	13.6
9	do.	1,800	4.4	9.4	.7	14.5
10	do.	900	2.7	9.7	1.3	13.7
L.S.D. at the 5-percent level.....			2.1	2.1	0.5	2.0
L.S.D. at the 1-percent level.....			2.8	2.9	.6	2.7

with yields in most treated plots significantly higher than in the check plots, except at the low levels of both Krilium formulations and in the intermediate level of Krilium 6. The mean significant differences in tuber yields range from approximately 2.5 to 4.5 hundredweights to the acre. Table 2 further shows that there was a significant tendency in some of the conditioner-treated plots to produce larger tubers. These larger tubers were as a rule well-shaped with a mean diameter of over 2 inches on the longer axis.

TABLE 3.—*Yield of stringless beans on Juncos clay as affected by treatment with 3 soil conditioners used at 3 levels*

Treatment identification No.	Treatment		Yield per acre at indicated picking			Total
	Conditioner	Rate per acre	1st.	2nd.	3d.	
		Lb.	*Cwt.*	*Cwt.*	*Cwt.*	*Cwt.*
1	Check	—	46.4	104.0	25.9	176.3
2	Krilium 9	3,600	52.1	102.5	18.0	172.6
3	do.	1,800	46.0	99.9	24.9	170.8
4	do.	900	39.2	93.2	25.4	157.8
5	Krilium 6	3,600	44.6	102.0	27.0	173.6
6	do.	1,800	64.0	107.0	18.6	189.6
7	do.	900	43.7	98.6	26.0	168.3
8	Aerotil	3,600	48.2	103.3	22.0	173.5
9	do.	1,800	46.5	92.2	26.2	164.9
10	do.	900	62.5	102.8	17.5	182.8
L.S.D. at the 5-percent level			15.5	16.1	9.3	22.2
L.S.D. at the 1-percent level			20.8	21.5	12.4	29.7

STRINGLESS-BEAN YIELDS

The tests with stringless beans were made to determine the influence of soil-conditioner treatment on a plant of a different kind. In other areas it has been shown that not all crops respond to conditioner treatment (*4*). The beans were planted shortly after harvesting the potatoes. The mean crop yields are shown in table 3. There were no significant differences in total yields. The treatments had no effect on bean-crop maturity either.

DISCUSSION

Previous work by Jefferies, *et al.* (*3*), has indicated that Juncos clay contains slightly over 20 percent of free oxides and organic matter. This perhaps may explain the rather high percentage of water-stable aggregates in the untreated soil, as reported in table 1. Increases as high as 97 percent were obtained with the treatments in individual plots. Potato tuber pro-

duction was definitely affected in a beneficial way by the increased stability of aggregates obtained from treatment. The increases in yields ranged from almost 20 to 40 percent in extreme cases. These yield increases are not exciting in view of the extremely high expenses that farm-scale application of the conditioning chemicals would entail at current prices. Furthermore, even if some of these conditioners have been observed to have persistent effects on the soil (*2*) for more than 2 or maybe 3 years in cooler regions, it is very possible that their action would decrease with time in warmer areas.

The lack of response of the bean, in contrast to the potato crop, to improved soil-aggregate stability stresses the importance of plant differences in assessing soil characteristics in experimental work. All crops do not respond in similar fashion to changed physical conditions in the soil. What might prove useful for a given crop might be worthless for another.

Under present conditions it seems worthwhile to study the possibilities of using cheaper materials such as filter-press cake, bagasse, sawdust, and crop residues for the improvement of soil structural conditions rather than using the costly synthetic polymers.

SUMMARY

Data are presented here on the effect of the synthetic soil conditioners Krilium (formulations 6 and 9) and Aerotil on the aggregate stability of Juncos clay and on the yield of potatoes and string beans.

The conditioners used were formulations 6 and 9 of Krilium, dry form, and Aerotil, wettable flakes, each at rates of 900, 1,800, and 3,600 pounds to the acre.

At all levels Krilium 6 showed the highest aggregate-stabilizing capacity. Significant and highly significant differences were obtained between the stability of soil aggregates in the check plots and in all conditioner-treated plots, except where Krilium 9 was used at the minimum level. The production of potatoes of the Kennebeck variety was significantly increased by conditioner treatment, except at the low levels of both Krilium formulations and the intermediate level of formulation 6. Stringless beans did not respond to the increased stability of soil aggregates, which stresses the importance of recognizing crop differences in assessing soil characteristics.

RESUMEN

Se presentan aquí datos sobre el efecto de tres acondicionadores sintéticos de suelos en la estabilidad de los agregados, o unidades estructurales del suelo Juncos arcilloso y en los rendimientos de papas y habichuelas sin fibra.

Los acondicionadores probados fueron Krilium 6 y 9 y Aerotil, cada uno

a razón de 900, 1,800 y 3,600 libras por acre. El Krilium 6 fué el mejor agente estabilizador de agregados. Se obtuvo diferencias significativas entre la estabilidad de los agregados en los suelos de las parcelas testigos y en los de las parcelas que recibieron tratamiento químico, excepto donde se usó la cantidad mínima de Krilium 9. La producción de papas de la variedad Kennebeck aumentó significativamente con los tratamientos, excepto con los niveles bajos de Krilium 6 y 9 y con el intermedio de Krilium 6. Los rendimientos de habichuelas sin fibra no se afectaron significativamente como resultado de los efectos de los tratamientos en la estabilidad de los agregados del suelo. Esto señala la necesidad de reconocer las diferencias entre las plantas al evaluar las propiedades del suelo.

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Bound Ascorbic Acid in Acerola Juice[1, 2]

Alice del Campillo and Conrado F. Asenjo[3]

INTRODUCTION AND OBJECTIVE

The unusually high content of ascorbic acid in acerola juice (*1*, *2*, *3*)[4] suggests that some of it may occur in the bound form. Consideration of this possibility resulted in the present investigation of the chemical state of ascorbic acid in that juice.

LITERATURE REVIEW

McHenry (*4*) and Ahmad (*5*) were the first to point out the possible presence of a combined form of ascorbic acid in plant tissue. Precipitates containing bound ascorbic acid were obtained by Reedman and McHenry from plant extracts (*6*). Holtz and Walter (*7*) determined bound ascorbic acid in animal tissue. Since then much work has been done on bound ascorbic acid and it has been the subject of considerable controversy, as many investigators have questioned the existence of a bound form of this vitamin.

Sumerwell and Sealock (*8*) developed a method that established the presence of appreciable amounts of bound ascorbic acid in liver tissue. Their results were verified in this laboratory by Jeffay (*9*). According to the technique of Sumerwell and Sealock (*8*), bound ascorbic acid is the ascorbic acid not extracted by 95-percent ethanol, and which can be determined only after the ethanol-insoluble fraction is subjected to acid hydrolysis.

METHODS

The method of Sumerwell and Sealock (*8*), with some modifications in order to adapt it to the acerola juice, was used. It consists in extracting the free ascorbic acid in the juice with 3 volumes of ice-cold ethanol in an atmosphere of carbon dioxide. After washing the precipitate nine times with ethanol, it was hydrolyzed in 5-percent metaphosphoric acid to release the bound ascorbic acid. The hydrolysis was accomplished by adding 1.5 volumes (with respect to the original volume of juice) of 5-percent metaphosphoric acid and heating the mixture for 15 minutes in a boiling water

[1] This investigation was made possible by a grant made to one of us (C.F.A.) by the Guggenheim Foundation.

[2] A preliminary report of this work was presented by the authors at the meeting of the Federation of Biological Societies in Atlantic City, Apr. 16–20, 1956.

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[4] Italic numbers in parentheses refer to Literature Cited, p. 139.

bath while passing carbon dioxide. After cooling, the mixture was diluted with 5-percent metaphosphoric acid to a definite volume, filtered, and the ascorbic acid liberated determined.

The ascorbic acid was determined by the routine method of Roe and Kuether (*10*). The ascorbic acid was first oxidized to dehydroascorbic acid with saturated bromine water, as described by Roe and Oesterling (*11*), until a faint, permanent yellow color was produced. The color of the excess bromine was removed by aeration or by adding a few drops of a solution of 10-percent thiourea in 50-percent ethanol. The dehydroascorbic acid was then coupled with 2,4-dinitrophenylhydrazine to form the osazone, following the method of Roe and Kuether (*10*). The osazone thus formed reacts with 85-percent sulfuric acid to give a red-orange complex. The intensity of the color of this complex was read against a reagent blank in the Evelyn photoelectric colorimeter using filter No. 54.

The micromethod of Bessey (*12*) for ascorbic and dehydroascorbic acid was also used with some modifications. In order to convert any dehydroascorbic acid to ascorbic acid the samples were treated with hydrogen sulfide. The excess hydrogen sulfide was removed by gassing extensively with wet-tank nitrogen. The measurements were made in a Beckman DU-Spectrophotometer at 518 mμ. The method consists in following the decrease in absorption at the indicated wavelength, when a solution of ascorbic acid is added to a solution of 2,6-dichlorophenolindophenol at a pH of 3.7. The decrease in absorption observed in the first 15 seconds was used for the calculation of the amount of ascorbic acid present in the sample. A solution of known content of ascorbic acid was used as reference.

RESULTS

EXTRACTION OF FREE ASCORBIC ACID

The results of a typical extraction procedure appear in table 1. To 10 ml. of acerola juice containing about 100 mg. of ascorbic acid, 30 ml. of ice-cold 95-percent alcohol were added. Immediately a precipitate appeared which was centrifuged off. A large proportion of the free ascorbic acid present in the juice was removed by this first ethanol extraction. Although practically all the free ascorbic acid had been removed after the fifth extraction with 30-ml. volumes of ethanol, the precipitate was still washed five more times. The last two washings contained practically no ascorbic acid. However, in order fully to rule out the possibility that the precipitate could have occluded free ascorbic acid, it was extracted in a Potter-Elvehjem homogenizer with ethanol, at room temperature, for 5 to 10 minutes. The alcoholic extract thus obtained contained no ascorbic acid, as determined by the 2,4-dinitrophenylhydrazine method. Yet the residue left behind yielded additional ascorbic acid after acid hydrolysis.

LIBERATION OF ASCORBIC ACID FROM THE PRECIPITATE

The precipitate was hydrolyzed at 100° C. with 5-percent metaphosphoric acid by the procedure already described. It contained 0.19 percent

TABLE 1.—*Results of using the typical extraction procedure described in the text on juice of the acerola*

Treatment	Extraction No.[1]	Total ascorbic acid
		Milligrams
Extraction of free ascorbic acid[2]	1	99.3
	2 }	
	3 }	5.0
	4 }	
	5 }	.14
	6 }	
	7 }	.06
	8 }	
	9	.0015
	10	.0015
Precipitate homogenized[3]	11	0
Bound ascorbic acid[4]	([5])	.19

[1] The braces indicate that extractions 2 and 3; 4 and 5; and 6, 7, and 8 were combined into 1 sample each before the ascorbic acid assay was performed.

[2] Extraction of free ascorbic acid from 10 ml. of acerola juice with ethanol.

[3] Precipitate extracted in a homogenizer.

[4] Bound ascorbic acid liberated from precipitate by acid hydrolysis.

[5] Acid hydrolysis of precipitate.

TABLE 2.—*Total and bound ascorbic acid found present in 6 samples of acerola juice*

Sample No.	Juice	Total ascorbic acid present	Bound ascorbic acid present	Proportion bound
	Milliliters	*Milligrams*	*Milligrams*	*Percent*
1	10	100	0.15	0.15
2	10	100	.16	.16
3	10	100	.19	.19
4	100	1,000	2.3	.23
5	200	2,000	3.3	.17
6	200	2,000	3.5	.18

of the total ascorbic acid in the juice. In table 2 are recorded the results obtained in six determinations of different samples of ripe acerola juice. The percentage of bound ascorbic acid ranged from 0.15 to 0.23, averaging 0.18 percent of the total ascorbic acid present in the juice.

If the hydrolysis were carried out at room temperature (27° C.) for the

same period of time, only 43 percent of the bound ascorbic acid found was liberated.

AMOUNT AND NATURE OF PRECIPITATE CONTAINING BOUND ASCORBIC ACID

The yield of the precipitate containing bound ascorbic acid ranged between 0.19 and 0.23 percent, averaging 0.22 percent by weight of juice as indicated in table 3. The juice from acerolas kept frozen for several months, as well as canned juice, were found to contain about the same amount of bound ascorbic acid as the fresh acerola juice.

The precipitate was insoluble in water and contained from 1.0 to 1.5 percent of nitrogen. It did not contain sulfur or halogens. Qualitative tests for proteins such as biuret, ninhydrin, and Millons were negative; the xanthoproteic test, however, was positive. The precipitate gave a positive Molisch test, but a negative starch-iodine test. The Benedict test for reducing sugars was positive only after acidification and neutralization, indicating that the liberated ascorbic acid was probably the responsible reducing agent.

TABLE 3.—*Weight and yield of precipitate containing bound ascorbic acid obtained from 4 samples of acerola juice*

Sample No.	Juice	Weight of juice	Weight of precipitate	Yield
	Milliliters	*Grams*	*Milligrams*	*Percent*
1	10	10.4	19.5	0.19
2	100	104.0	236.0	.23
3	200	208.0	455.0	.22
4	200	208.0	480.0	.23

CHARACTERIZATION OF LIBERATED ASCORBIC ACID

Attempts to isolate the ascorbic acid osazone prepared from the ascorbic acid released from the bound complex were not altogether successful. The method of Sumerwell and Sealock (*8*) was used and it was possible to follow the formation of the osazone in the incubation mixture. However, the solid obtained, which should have been the ascorbic acid osazone, had an indefinite melting point due to impurities. Attempts to purify this precipitate were unsuccessful because the amount available was very small. After two reprecipitations the material showed charring at 200° C.

The red-orange-colored complex formed by the reaction of the dehydroascorbic acid osazone with 85-percent sulfuric acid was used to characterize the liberated ascorbic acid. The absorption spectrum of this red-orange complex after dilution in a 1:1 ethanol-acetone mixture was determined

in a Beckman DU-Spectrophotometer. The blank cell contained the same reagents as the experimental cell, the only difference being the order in which the reagents were added. Figure 1 shows the absorption curves obtained with pure ascorbic acid and with ascorbic acid released by acid hydrolysis of the solid fraction. Both curves are very similar with a maximum at 440 mμ.

In order to provide additional evidence that the reducing agent liberated after acid hydrolysis of the precipitate was ascorbic acid, this was determined in the same sample by the 2,4-dinitrophenylhydrazine method and by the Bessey micromethod (*12*) using 2,6-dichlorophenolindophenol. The

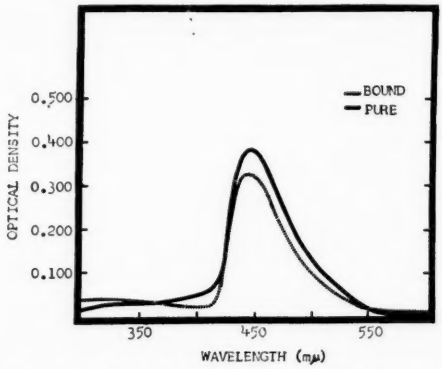

FIG. 1.—Absorption curves obtained with pure ascorbic acid and with ascorbic acid released by acid hydrolysis of the solid fraction.

yield of ascorbic acid by both methods was practically identical: 8.00 μg./mg. and 8.07 μg./mg., respectively.

SUMMARY

1. Acerola juice, fresh, frozen, or canned, was found to contain in the neighborhood of 0.22 percent by weight of an alcohol-insoluble precipitate containing bound ascorbic acid. This precipitate contained about 1.25 percent of nitrogen.

2. Of the total ascorbic acid in acerola juice 0.18 percent was in the bound form.

3. The bound ascorbic acid liberated was characterized by means of the absorption spectrum curve of its osazone-sulfuric acid complex.

RESUMEN

1. El jugo de acerola fresco, congelado o enlatado contiene alrededor de 0.22 por ciento por peso de un precipitado insuluble en alcohol que en si

contiene ácido ascórbico conjugado. Este precipitado contiene cerca de 1.25 por ciento de nitrógeno.

2. Del total de ácido ascórbico en el jugo de acerola, 0.18 por ciento se encuentra en este estado conjugado.

3. El ácido ascórbico conjugado se liberó y se caracterizó por medio de la curva de absorción de su complejo osazona-ácido sulfúrico.

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The Effect of the Presence of Different Concentrations of Fusel Oil on the Determination of Alcohol by Picnometer and Hydrometer

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INTRODUCTION

During the course of operations in a commercial distillery it is frequently necessary to determine the ethyl alcohol content of samples from intermediate plates of the distillation columns which contain relatively large amounts of fusel oil and smaller quantities of other impurities. It is desirable to make this determination without resorting to time-consuming and complicated methods of analysis which are not regularly utilized in daily process control. Determination of the alcohol being removed from the column with the fusel-oil side stream, and establishing the relation between alcohol and fusel-oil compositions along the column are examples of this need.

The methods of analysis most commonly employed in distillery practice for the determination of alcohol are those of the picnometer, the refractometer, and the hydrometer. The last is by far the most used. The picnometer and hydrometer methods are based on the specific gravity of pure alcohol-water solutions at reference temperatures, while the refractometer method is based on the index of refraction of pure solutions, also at reference temperatures.

Obviously, the presence of substances other than alcohol and water will alter the physical properties of the solution, introducing errors in the results obtained with any of the above analytical methods. Fortunately, the quantities of impurities in the raw products of the first columns of the distillation unit, and especially in the finished product from the last column, are so low that the error introduced is usually negligible and falls well within the precision limits of the various analytical methods. In aged spirits and finished beverage products the effect of impurities is minimized by distilling the sample in the laboratory following established procedures before measuring its specific gravity or refractive index.

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When dealing with samples from intermediate plates of the rectifying column, in which the alcohol content is relatively low while the fusel-oil content could be 10 to 20 percent, and in some cases higher, a simple distillation of the sample would be ineffective. Even careful fractionation of the sample would not be satisfactory, since a substantial part of the fusel oil would pass into the overhead product if all of the ethyl alcohol present in the sample is to be recovered in the distillate. The difficulties encountered in the separation of fusel oil-alcohol-water by distillation have been discussed elsewhere (*1*).[2]

OBJECTIVE

This work was undertaken with the purpose of providing a method for correcting for the error introduced by the presence of different concentrations of fusel oil at different alcoholic strengths.

EXPERIMENTAL PROCEDURES

Solutions of known alcohol and fusel-oil contents were prepared and analyzed for apparent alcoholic content by means of the picnometer and hydrometer. Solutions containing 200, 500, 1,000, 2,000, 4,000, 7,000, 10,000, 20,000, and 30,000 mg. of fusel oil as amyl alcohol per 100 ml. of sample, at 10, 20, 30, 40, 50, 60, 70, 80, and 90 percent of alcohol by volume at 60° F. were prepared. The absolute alcohol used as source of ethanol was analysed in triplicate by the picnometer method and found to contain 99.25 percent of alcohol by volume at 60° F.

Commercial fusel oil from a Puerto Rican distillery was used as the source of fusel oil. The oil had been concentrated by extraction with water at its origin plant, and contained about 60 percent of fusel oil. It was further extracted twice, using one-half its own volume each time of water saturated with commercial table salt. After thorough mixing, the mixture was allowed to separate during several hours and the oily upper layer was decanted. The oil was then fractionally distilled at atmospheric pressure in a 1-inch glass column, 6 feet in height, packed with glass Raschig rings. Portions of 1,500 ml. were placed in a 2.5-l. flask and heat was supplied by a constant-temperature mineral-oil bath. The temperature of the oil bath was regulated to maintain an overhead rate of 30 ml. per hour at a reflux ratio of 10:1. The reflux ratio was automatically regulated by an electric timer and a magnetically operated flow-divider in the glass column head. The column was maintained at total reflux for a period of 2 hours before removal of the condensate began.

A first fraction of 150 ml., boiling between 83° and 87° C. was discarded to insure complete removal of any ethyl alcohol present. In doing so water and some of the lower homologues of the higher alcohols were also removed.

[2] Italic numbers in parentheses refer to Literature Cited, p. 145.

A second fraction of 1,150 ml., boiling between 87° and 132° C., was collected as purified fusel oil.

A third fraction, consisting of the residue remaining in the flask, was stored apart. After there was sufficient residue, 1,500 ml. were placed in the flask and fractionated as before, except that no first fraction was discarded. The residue was evaporated practically to dryness at 136° C. The product of this distillation was mixed with the fusel-oil fraction collected in the first fractionation. The oil mixture was analyzed in triplicate for specific gravity and fusel-oil content giving specific gravity values of $0.8212^{25°C.}_{25°C.}$ and 86,665 mg. of fusel oil as amyl alcohol per 100 ml. The apparent discrepancy of obtaining over 100 percent of fusel oil by weight occurred because the fusel-oil content is expressed as amyl alcohol while it actually consists of a mixture of alcohols containing appreciable portions of lower molecular-weight homologues. These figures are indicative of the high degree of purity of the fusel oil obtained.

The samples were prepared by pipetting previously calculated quantities of purified fusel oil and absolute alcohol into 250-ml. calibrated volumetric flasks and completing to volume with distilled water. Sixty-milliliter glass picnomeers provided with individual thermometer-caps were utilized. The calibration of the hydrometers used was checked with pure water-alcohol solutions of known composition.

RESULTS

The analytical results of this work are presented in table 1. It can be observed that numerous samples were discarded because of the presence of two liquid-phase layers.

The results show that the differences between apparent and true alcohol content obtained by the hydrometer and picnometer methods were almost identical throughout the range of alcoholic strength and fusel-oil content studied. This excellent agreement is to be expected since both methods are based on the specific gravity of the sample. The difference between apparent and true alcohol content by both methods varied with the alcoholic strength at any given fusel-oil concentration. At lower alcohol contents the error increased as the alcoholic strength increased, and after passing a maximum it decreased with further increase in alcohol content.

With the higher fusel-oil concentrations the maximum is not shown since partial solubility exists at the lower alcoholic strength and two liquid phases are formed. This variation is due to the combination of contraction and concentration effects. The error decreases as the alcohol-water content is such that its specific gravity approaches that of the fusel oil. If fusel oil with a specific gravity of $0.8212^{25°C.}_{25°C.}$ is added to an alcohol-water solution of that same specific gravity, which corresponds to an alcoholic strength of

approximately 185° proof, no error would be introduced in the determination of alcohol by the picnometer and hydrometer methods, should the con-

TABLE 1.—*Effect of fusel oil on the determination of alcohol by picnometer and hydrometer*[1]

Fusel oil, (mg. per 100 ml. of sample)	Analytical method	Actual alcohol content at which samples were prepared (degrees proof at 60° F.)								
		20° P.[2]	40° P.[2]	60° P.	80° P.	100° P.	120° P.	140° P.	160° P.	180° P.
200	Picnometer	20.4	40.1	59.8	79.9	100.1	119.9	140.0	160.1	180.2
	Hydrometer	20.2	40.0	59.2	80.0	99.0	120.0	140.1	160.4	180.4
500	Picnometer	20.7	40.6	60.8	80.8	100.8	120.9	140.8	160.4	180.8
	Hydrometer	20.6	40.4	60.2	80.6	100.4	120.8	140.8	160.9	180.4
1,000	Picnometer	21.5	41.7	62.0	82.2	102.2	122.1	142.1	162.2	182.1
	Hydrometer	21.8	41.6	61.8	82.4	102.4	122.3	142.4	162.6	182.6
2,000	Picnometer	23.3	43.8	64.4	84.5	104.6	124.4	144.4	164.1	184.0
	Hydrometer	23.6	43.8	64.9	84.1	104.8	124.6	144.3	164.4	184.2
4,000	Picnometer		48.4	([3])	89.8	109.6	128.9	148.7	168.5	188.0
	Hydrometer		47.8	([3])	89.2	109.4	129.1	149.0	168.7	188.4
7,000	Picnometer			77.4	97.2	116.2	135.7	155.2	174.6	—
	Hydrometer			77.2	97.0	115.9	135.3	155.7	174.4	—
10,000	Picnometer			85.4	104.5	123.3	142.4	161.5	181.0	—
	Hydrometer			84.8	104.1	123.1	142.4	161.7	181.5	—
15,000	Picnometer			97.7	115.9	134.3	153.3	172.1	190.6	—
	Hydrometer			97.2	115.3	134.3	153.8	172.2	190.8	—
20,000	Picnometer			109.0	126.5	144.8	163.1	181.7	—	—
	Hydrometer			108.6	126.4	144.8	163.2	182.0	—	—
30,000	Picnometer			129.7	146.7	164.3	182.6	—	—	—
	Hydrometer			129.6	146.9	164.6	182.7	—	—	—

[1] Analytical results in degrees proof at 60° F.
[2] Two liquid layers formed when blanks occur in this column.
[3] Sample discarded.

traction effects be negligible. Accordingly, at any given fusel-oil concentration the error would tend to decrease as the alcohol content is increased. However, the results obtained indicate that this effect is more than offset at the lower alcoholic content, probably by an expansion effect, and the error increases with increased alcohol content up to a maximum which de-

pends on the fusel-oil content. After this maximum the error decreases with increased alcoholic strength.

The experimental results obtained by the picnometer method have been plotted in figure 1. This graph allows rapid determination of the correction to be introduced if the fusel-oil content and the apparent proof of the sample are known. The results indicate that, at any true alcoholic strength, the error produced is directly proportional to the fusel-oil content. This fact

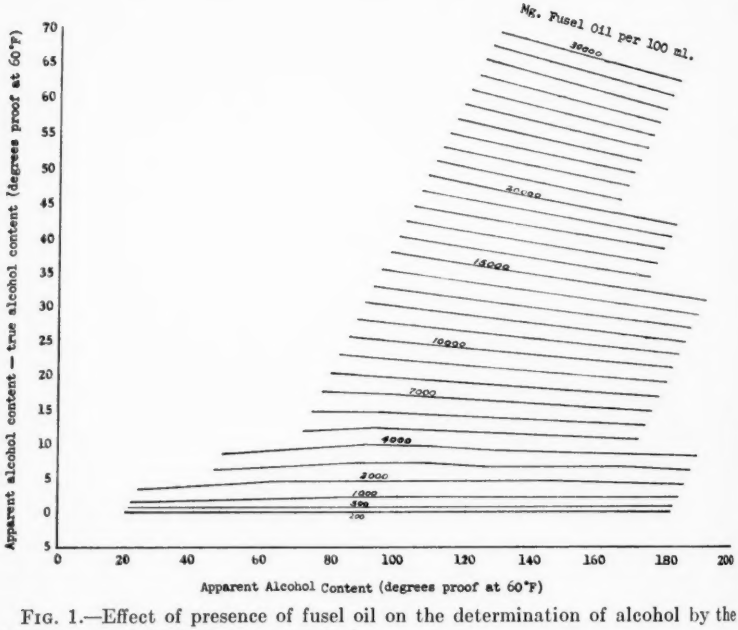

FIG. 1.—Effect of presence of fusel oil on the determination of alcohol by the picnometer and the hydrometer methods.

allows direct interpolation between the lines through experimental points of various fusel-oil concentrations. Since, in figure 1, apparent proof as abscissa has been plotted versus apparent proof-true proof as ordinate, the points of constant true alcohol content fall on a straight line the slope of which is 1 when equal scales are used in the abscissa and in the ordinate. In our case the ordinate scale is twice as large as the abscissa scale and, therefore, the family of lines of fixed true alcoholic content has a slope of 2. By interpolating along these lines between different fusel-oil contents, lines have been drawn in figure 1 at concentration intervals of 1,000 mg. of fusel oil as amyl alcohol per 100 ml. of sample.

The fusel-oil-content lines can be extrapolated, if necessary, to cover values of apparent alcohol outside the range covered by the experimental values plotted. It is believed that true alcoholic strengths can be determined with the aid of figure 1 within an accuracy of about 1-percent alcohol by volume at 60° F. A large-size plot would improve the accuracy. Figure 1 can be used for determining correction values for both the picnometer and the hydrometer methods since, as was stated previously, the experimental values obtained by both methods for the deviations caused by the presence of different concentrations of fusel oil are in excellent agreement.

Fusel-oil concentrations up to 200 mg. of fusel oil per 100 ml. of sample introduce negligible errors at any true alcoholic strength with the analytical methods studied.

SUMMARY

Based on experimental data, a graph has been prepared which allows rapid determination of the correction to be introduced in order to determine the true alcoholic content of a sample, if its fusel-oil content is known and the apparent alcoholic strength has been determined by the hydrometer or picnometer.

RESUMEN

Basado en datos experimentales, se ha desarrollado una gráfica de la cual puede obtenerse rápidamente la corrección que deberá usarse para obtener el contenido alcohólico verdadero de una muestra, si se conoce su contenido de aceite de fúsel y si su grado prueba aparente ha sido determinado por los métodos de alcoholómetro o picnómetro.

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(Continued from inside front cover)

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[1] As of April 1, 1957.
[2] Leave to pursue studies.
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